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Notes on a Rare Bornean Bufonid *Ansonia latidisca* Inger, 1966, with Special Reference to Its Phylogenetic Position

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Abstract: On the basis of three female specimens collected recently, some aspects of a little known Bornean bufonid, *Ansonia latidisca*, are reported. On the mitochondrial phylogenetic tree, the species is basal to the group consisting of some Bornean species, and most Peninsular Malaysian and Thailand species, which is sister to the other group consisting of the remaining Bornean species, several Philippine species, and one Peninsular species. This relationship indicates that the the genus *Ansonia* has originated within Borneo. Superficial similarity of the species with *Sabahphrynus maculatus* is thought be the result of convergence in adaptation to an arboreal life.

Key words: *Ansonia*; Borneo; Phylogeny; Rare species; *Sabahphrynus*

INTRODUCTION

Oriental stream toads of the genus *Ansonia* Stoliczka, 1870 are famous for their unique larvae that adhere to rocks in fast-flowing, high gradient streams by a large oral sucker (Inger, 1966; Matsui et al., 2005). The genus encompasses about 26 described species (Frost, 2011; Wilkinson et al., 2012), with several additional taxa still requiring formal descriptions (Matsui et al., 2010). Among already named congeners, *A. latidisca* Inger, 1966 from the western part of Borneo is one of the least known species.

The species was described based on the male holotype (RMNH [Rijksmuseum van Natuurlijke Historie=The National Museum of Natural History "Naturalis" in Leiden] 10677) from top of Mount Damus, Sambas, Kalimantan, Indonesian Borneo, by J. G. Hallier (Inger, 1966; Gassó Miracle et al., 2007) and a female paratype (BMNH [British Museum of Natural History]=NHM [Natural History Museum], London 99.12.8.12) from Mount Penrissen, First Division (now Kuching Division), Sarawak, Malaysian Borneo (Inger, 1966).

The holotype seems to have been collected in 1893 (Fig. 1, see discussion), and from its catalogue number, the paratype should have been collected before 1899, both more than 100 years ago. Another specimen (NHM, London 1973.528 [Indraneil Das, personal communication on 10 September 2012]) is said to have been collected in 1924 (Conservation

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FIG. 1. The male holotype of *Ansonia latidisca* (RMNH 10677) deposited in The National Museum of Natural History "Naturalis" in Leiden.

International, 2011), but further detailed data are not available to us at present. Because the habitats at least in Sarawak have been seriously modified, there has been serious concern about the extinction of the species there. For example, *A. latidisca* has been listed as Endangered B1ab(iii)+ 2ab(iii) by IUCN (Stuart et al., 2008). However, in the course of the "Global Search for Lost Amphibians" by IUCN, the species was finally rediscovered in 2011 on Mount Penrissen, Sarawak (Conservation International, 2011). We also made a survey there twice recently and were able to find the species ourselves.

From examination of the holotype in the Rijksmuseum (Matsui, unpublished observations) and from description in the literature, we had the impression that the species superficially resembles *Sabahphrynus maculatus* (Mocquard, 1890) from Sabah, Malaysian Borneo (Matsui et al., 2007), and have been interested in elucidating the phylogenetic position of *A. latidisca* among *Ansonia* and allied genera. We therefore studied the specimens obtained by analyzing mitochondrial gene sequences, based on the relationships of most members of the genus *Ansonia* that have already been clarified (Matsui et al., 2010).

MATERIALS AND METHODS

Fieldwork was conducted in September 2010 and February to March 2012 on Gunung (=Mt.) Penrissen, Padawan, western Sarawak. Specimens are deposited in the Sarawak Research Collections (SRC) and the Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

The following 25 body measurements were taken to the nearest 0.1 mm with dial calipers, following Matsui (1984) and Matsui (1994): (1) snout-vent length (SVL); (2) head length (HL); (3) snout length (SL); (4) nostril-eyelid length (N-EL); (5) eye length (EL); (6) eye diameter (ED), diameter of the exposed portion of the eyeball; (7) tympanum-eye length (T-EL); (8) tympanum diameter (TD); (9) head width (HW); (10) internarial distance (IND); (11) interorbital distance (IOD); (12) upper eyelid width (UEW); (13) forelimb length (FLL); (14) lower arm and hand length (LAL); (15) hand length (HAL); (16) inner palmar tubercle length (IPTL); (17) outer palmar tubercle length (OPTL); (18) hindlimb length (HLL); (19) thigh length (THIGH); (20) tibia length (TL); (21) foot length (FL); (22) inner metatarsal tubercle length (IMTL); (23) outer metatarsal tubercle length (OMTL); (24) third finger disk diameter (3FDW); and (25) fourth toe disk diameter (4TDW). For morphological comparisons, we also examined specimens of *Sabahphrynus maculatus* deposited in the KUHE.

We examined DNA sequences of 12S and 16S rRNA genes and the intervening tRNA gene for valine from 25 specimens of 23 named species (including three individuals of *A. latidisca*) of the genus *Ansonia*. We also examined the sequences of representatives of

five other Southeast Asian bufonid genera (*Sabahphrynus* Matsui, Yambun, and Sudin, 2007; *Pedostibes* Günther, 1876; *Pelophryne* Barbour, 1938; *Leptophryne* Fitzinger, 1843; and *Bufo* Laurenti, 1768 [sensu lato]), and two distinctly distant (Frost et al., 2006) outgroup species (a bufonid, *Atelopus flavescens* Duméril and Bibron, 1841 and a dendrobatid *Dendrobates auratus* [Girard, 1855]: Table 1).

Methods for phylogenetic analyses follow

TABLE 1. Samples used for mtDNA analysis in this study together with information on species identification, locality, GenBank accession numbers, and references. KUHE=Graduate School of Human and Environmental Studies, Kyoto University.

Species	Locality	Data Bank Acc. No.	Reference
Ansonia kraensis	Thailand, Ranong	AB435251	Matsui et al. (2010)
Ansonia inthanon	Thailand, Doi Inthanon	AB435253	Matsui et al. (2010)
Ansonia siamensis	Thailand, Khaochong	AB435256	Matsui et al. (2010)
Ansonia endauensis	West Malaysia, Johor, Endau-Rompin	AB435257	Matsui et al. (2010)
Ansonia tiomanica	West Malaysia, Pahang, Tioman	AB435259	Matsui et al. (2010)
Ansonia latirostra	West Malaysia, Pahang	AB435260	Matsui et al. (2010)
Ansonia penangensis	West Malaysia, Penang	AB435262	Matsui et al. (2010)
Ansonia malayana	West Malaysia, Larut	AB331712	Matsui et al. (2010)
Ansonia jeetskumarani	West Malaysia, Pahang	AB435265	Matsui et al. (2010)
Ansonia platysoma	East Malaysia, Sabah, Bundu Tuhan	AB435270	Matsui et al. (2010)
Ansonia hanitschi	East Malaysia, Sabah, Kinabalu	AB435277	Matsui et al. (2010)
Ansonia spinulifer	East Malaysia, Sarawak, Kuching	AB435289	Matsui et al. (2010)
Ansonia minuta	East Malaysia, Sarawak, Kuching	AB435281	Matsui et al. (2010)
Ansonia latidisca	East Malaysia, Sarawak, Penrissen	AB746459	KUHE 55421
Ansonia latidisca	East Malaysia, Sarawak, Penrissen	AB746460	KUHE 55422
Ansonia latidisca	East Malaysia, Sarawak, Penrissen	AB746461	KUHE 55423
Ansonia longidigita	East Malaysia, Sabah, Crocker	AB331711	Matsui et al. (2010)
Ansonia torrentis	East Malaysia, Sarawak, Gn. Mulu	AB435296	Matsui et al. (2010)
Ansonia leptopus	East Malaysia, Sarawak, Kuching	AB746457	KUHE 53839
Ansonia latiffi	West Malaysia, Pahang	AB435299	Matsui et al. (2010)
Ansonia albomaculata	East Malaysia, Sarawak, Lanjak Entimau	AB435304	Matsui et al. (2010)
Ansonia guibei	East Malaysia, Sabah, Kinabalu	AB435306	Matsui et al. (2010)
Ansonia fuliginea	East Malaysia, Sabah, Kinabalu	AB435308	Matsui et al. (2010)
Ansonia muelleri	Philippines, Mindanao, Davao City	AB435310	Matsui et al. (2010)
Ansonia mcgregori	Philippines, Mindanao	AB435316	Matsui et al. (2010)
Sabahphrynus maculatus (=*Ansonia anotis*)	East Malaysia, Sabah, Kinabalu	AB331708	Matsui et al. (2010)
Sabahphrynus maculatus	East Malaysia, Sabah, Crocker	AB331718	Matsui et al. (2010)
Pelophylyne signata	East Malaysia, Sarawak, Kuching	AB746456	KUHE 53200
Bufo (*Ingerophryne*) *parvus*	West Malaysia, Penang	AB746455	KUHE 39047
Leptophryne borbonica	East Malaysia, Sarawak, Penrissen	AB746458	KUHE 53887
Bufo (*Duttaphrynus*) *melanostictus*	East Malaysia, Sarawak, Marudi	AB331714	Matsui et al. (2007)
Pedostibes hosii	East Malaysia, Sabah, Tawau	AB331717	Matsui et al. (2010)
Bufo (*Phrynoides*) *asper*	West Malaysia, Penang	AB746454	KUHE 39025
Atelopus flavescns	French Guiana	DQ283259	Frost et al. (2006)
Dendrobates auratus	—	AY326030	Darst and Cannatella, 2004

Matsui et al. (2010). The PCR cycling, precipitation, and sequencing procedures were identical to those described by Matsui et al. (2010). The resultant sequences were deposited in GenBank (AB746459–746461: Table 1). The alignment matrix with 2462 nucleotide sites (942 sites for 12S rRNA; 72 for $tRNA_{val}$; 1448 for 16S rRNA) was subjected to estimation of phylogenetic relationships using maximum likelihood (ML) and Bayesian inference (BI). In the BI analysis, two independent runs of four Markov chains were conducted for ten million generations, and the first three million generations were discarded as burn-in. Pairwise comparisons of uncorrected sequence divergences (p-distances) were also calculated for 16S rRNA. Details for these procedures are given in Matsui et al. (2010).

RESULTS

Natural History and morphology

We were only able to find three specimens of *A. latidisca* on one rainy night in late February. They were found in a narrow area in primary forest, on the slope surrounding a huge rocky mound (Fig. 2). A very slowly

FIG. 2. Natural habitat of *Ansonia latidisca* on Gunung Penrissen.

FIG. 3. *Ansonia latidisca* in life.

flowing headwater of a small stream, about 10 m away from the rock, was the nearest water body. Two individuals were on a leaf and the trunk, respectively, of huge trees (Fig. 3), and the remaining one was on a huge rock, all 1.5–2 m above ground. We could not hear any calls assignable to this species or find larvae in the water. Frog species observed immediately near the habitat were *Pelophryne* sp., *Limnonectes palavanensis* (Boulenger, 1894), *Limnonectes kuhlii* (Tschudi, 1838), and *Philautus refugii* Inger and Stuebing, 1996. A congeneric species *A. minuta* Inger, 1960 was found far down from there where the stream was flowing rapidly.

The three specimens obtained were all females. Two larger individuals (59.8 and 55.5 mm in SVL, Table 2) possessed small ovaries with creamy immature eggs, suggesting their non-breeding condition. The smallest female (52.1 mm in SVL) had more transparent ovaries and thus was thought to be immature. They are nearly uniform in body shape and coloration (Fig. 4), agreeing well with the diagnosis given in the original description of *A. latidisca* by Inger (1966): "a large species, females about 55 mm; tympanum visible externally; tips of fingers dilated into truncate disks (Fig. 5), that of third as wide as tympanum; tip of first finger not reaching disk of second; two rows of interorbital, conical tubercles; and no tarsal ridge". Inger's (1966) original description very well illustrates characteristics of the species and there is little to add:

Habitus slender; head, length (27.1–28.8% SVL) subequal to width (27.1–28.6%SVL); snout truncate, constricted before eyes, almost vertical in profile, projecting beyond lower jaw; eye small, subequal (10.0–11.0%SVL) to snout (9.8–10.8%SVL); canthus rostralis sharp, straight; lore vertical, weakly concave; nostril above symphysis, closer to tip of snout than to eye; interorbital distance (7.4–8.6% SVL) subequal to width of upper eyelid (7.7–7.9%SVL); the latter larger than width of internarial distance (6.3–7.0%SVL); pineal spot absent; tympanum distinct (4.6–5.6% SVL), about half diameter of eye; upper jaw edentate; tongue oval, without papillae.

Forelimb extremely long (78.3–86.8%SVL) and slender; fingers slender, long, with distinct web basally, web reaching subarticular tubercles of first two fingers; first finger short, not reaching base of disk of second; fourth finger longer than second; tips of three outer fingers dilated into truncate disks about twice width of basal phalanges; disk of third finger slightly narrower (4.0–5.0%SVL) than tympanum; a large, round palmar tubercle laterally (4.3–5.0%SVL) larger than outer (3.0–3.8%SVL); subarticular tubercles feebly distinct.

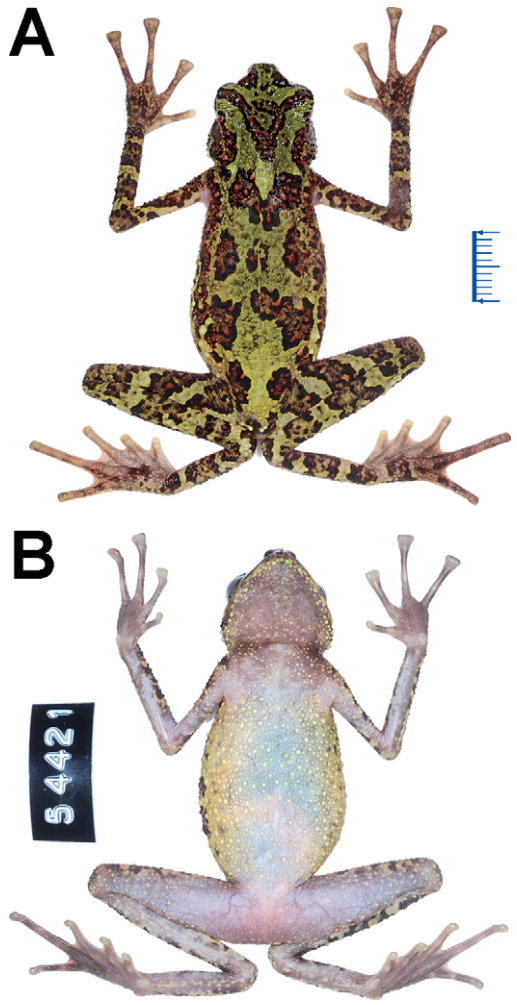

FIG. 4. Dorsal (A) and ventral (B) views, of *Ansonia latidisca*. Scale bar=10 mm.

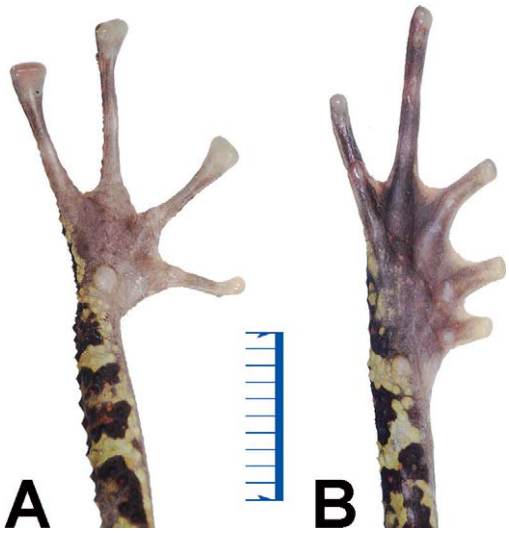

FIG. 5. Ventral views of right hand (A) and foot (B) of *Ansonia latidisca*. Scale bar=10 mm.

Hindlimb moderately long (139.8–160.1% SVL) less than times length of forelimb; tibia moderate (44.1–50.7%SVL), heels overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching to point between rear angle and center of eye, or between eye and nostril; foot (37.5–41.7%SVL) shorter than tibia; tips of toes swollen but not forming truncate disks, much narrower than tips of fingers (disk diameter of fourth toe 2.5–2.8%SVL); third toe shorter than fifth; webs between toes moderately developed (Fig. 4), formula: **I** 0–2 **II** 1–3 **III** 2–3 **IV** 3–2 **V**; subarticular tubercles obscure; inner metatarsal tubercle oval, length (4.8–5.0%SVL) shorter than first toe; outer metatarsal tubercle round, smaller (2.3–2.7% SVL) than inner one; no tarsal ridge.

Skin texture is slightly variable, but fits well with Inger's (1966) description. Inger (1966) noted that the color in alcohol of the holotype was light brown with numerous, irregular reddish brown spots dorsally and laterally; and ventrally, brown with scattered, small yellowish spots posteriorly. He also stated that the ventral color of the paratype was lighter (a brownish yellow or cream-color) than that of the holotype. Specimens in life were light greenish brown with irregular, large brown markings and scattered reddish brown spots dorsally and laterally. Ventrally, they were light brown on throat and cream with scattered, small yellowish spots posteriorly (Fig. 4).

Phylogeny

Of 2462 nucleotides generated, 1033 were variable, and 763 were parsimony-informative. The best substitution model for ML and

TABLE 2. Measurements (in mm, followed by percentage ratios to SVL) of three females of *Ansonia latidisca*. For abbreviations, see text. *now deposited at SRC.

KUHE	54422		54421		54423*		KUHE	54422		54421		54423*	
SVL	59.8		55.5		52.1								
HL	16.2	(27.1)	16.0	(28.8)	14.6	(28.0)	LAL	33.8	(56.5)	34.6	(62.3)	32.6	(62.6)
SL	5.4	(9.0)	6.0	(10.8)	5.2	(10.0)	HAL	17.0	(28.4)	16.7	(30.1)	16.4	(31.5)
N-EL	3.5	(5.9)	3.4	(6.1)	3.4	(6.5)	IPTL	2.6	(4.3)	2.8	(5.0)	2.5	(4.8)
EL	6.2	(10.4)	6.1	(11.0)	5.2	(10.0)	OPTL	2.3	(3.8)	2.1	(3.0)	2.0	(3.8)
ED	5.2	(8.7)	5.1	(9.2)	4.4	(8.4)	HLL	83.6	(139.8)	88.1	(158.7)	83.4	(160.1)
T-EL	0.5	(0.8)	0.8	(1.4)	0.5	(1.0)	THIGH	25.7	(43.0)	26.8	(48.2)	25.1	(48.2)
TD	3.0	(5.0)	3.1	(5.6)	2.4	(4.6)	TL	26.4	(44.1)	27.5	(49.5)	26.4	(50.7)
HW	16.2	(27.1)	15.9	(28.6)	14.4	(27.6)	FL	22.4	(37.5)	23.1	(41.6)	21.7	(41.7)
IND	4.2	(7.0)	3.6	(6.5)	3.3	(6.3)	IMTL	2.9	(4.8)	2.7	(4.9)	2.6	(5.0)
IOD	4.4	(7.4)	4.7	(8.5)	4.5	(8.6)	OMTL	1.4	(2.3)	1.5	(2.7)	1.3	(2.5)
UEW	4.6	(7.7)	4.3	(7.7)	4.1	(7.9)	3FDW	3.0	(5.0)	2.5	(4.5)	2.1	(4.0)
FLL	46.8	(78.3)	46.3	(83.4)	45.2	(86.8)	4TDW	1.7	(2.8)	1.4	(2.5)	1.4	(2.7)

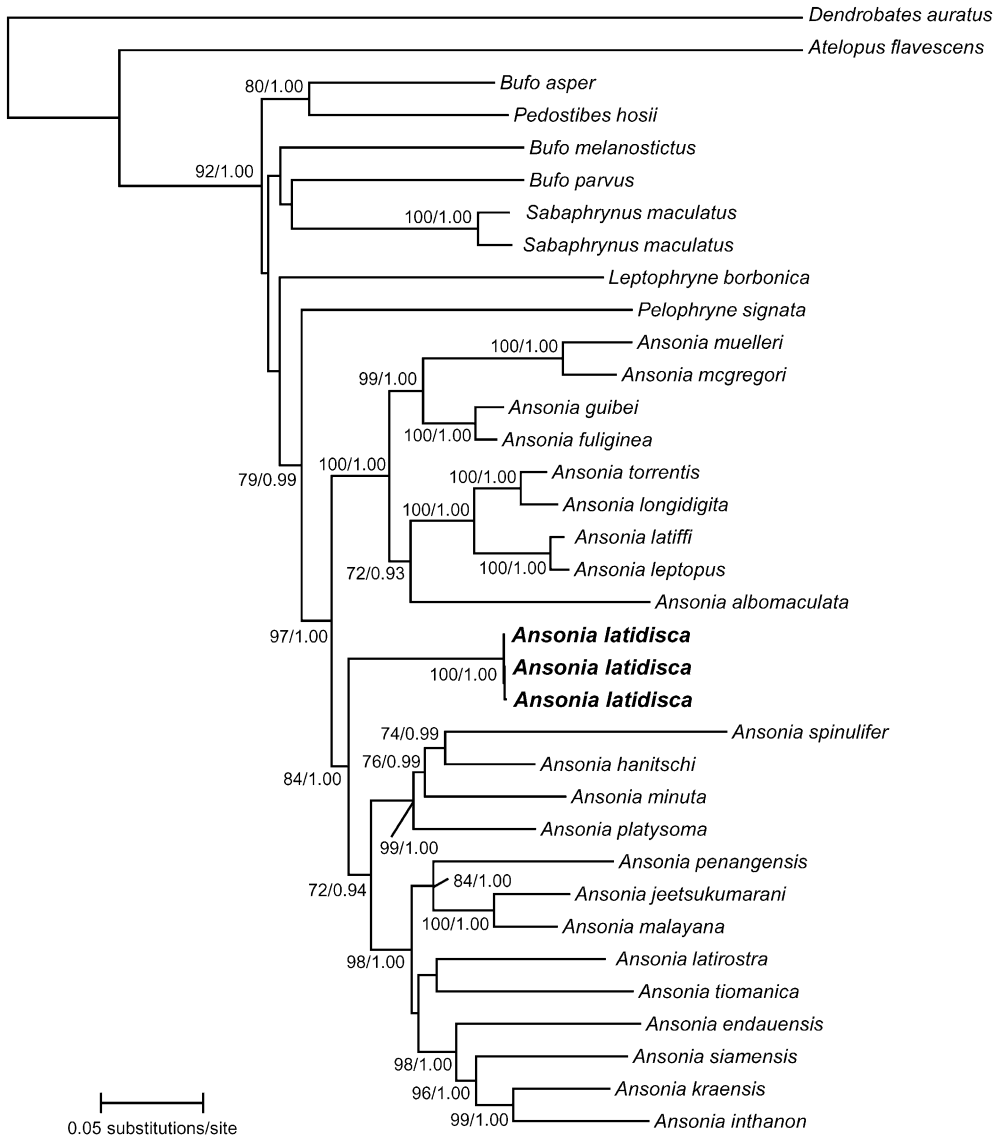

FIG. 6. ML tree from a 2462 bp sequence of mitochondrial 12S rRNA, tRNAval and 16S rRNA genes for samples of *Ansonia* and related species. Numbers above or below branches represent bootstrap supports for ML inferences and Bayesian posterior probabilities (ML-BS/BPP).

Bayesian inference derived from Kakusan4 (Tanabe, 2011) was a general time-reversible model with a gamma shape parameter (estimated gamma values for each analysis were 0.241 and 0.243, respectively). The likelihood value of the ML and Bayesian trees were lnL -22644.606 and -22682.499, respectively.

Phylogenetic analyses employing two different optimality criteria yielded very slightly different topologies, and only the ML tree is presented in Fig. 6. Monophyly of Southeast Asian bufonid taxa (*Bufo* [sensu lato], *Leptophryne*, *Pelophryne*, *Pedostibes*, *Sabahphrynus*, and *Ansonia*) with respect to *Atelopus* and *Dendrobates* was supported (ML BS=92%, BPP=1.00). Although relationships

among bufonid genera are generally unresolved, *Pelophryne* and *Ansonia* formed a clade (ML BS=79%, BPP=0.99), and the monophyly of *Ansonia* was strongly supported (ML BS=97%, BPP=1.00). As in a previous report (Matsui et al., 2010), *Ansonia* is clearly divisible into two sister clades. In one clade (ML BS=84%, BPP=1.00), *A. latidisca* was a sister species to Clade A of Matsui et al. (2010: ML BS=72%, BPP=0.94), including Subclade A1 (ML BS=98%, BPP=1.00) from Peninsular Malaysia and Thailand and Subclade A2 (ML BS=99%, BPP=1.00) from Borneo. The other was a primarily Bornean Clade B of Matsui et al. (2010: ML BS=100%, BPP=1.00), including Bornean and Philippine species, and one species from the Peninsular Malaysia. From the species of each clade and subclade, *A. latidisca* exhibited substantially large uncorrected p-distances in 16S rRNA of at least 9.3% (between *A. fuliginea* [Mocquard, 1890] in Clade B), 9.4% (between *A. minuta* in Sublade A2), and 9.9% (between *A. jeetsukumarani* Wood, Grismer, Ahmad, and Senawi, 2008 in Sublade A1), which indicate its intermediate position among the three *Ansonia* clades/subclades.

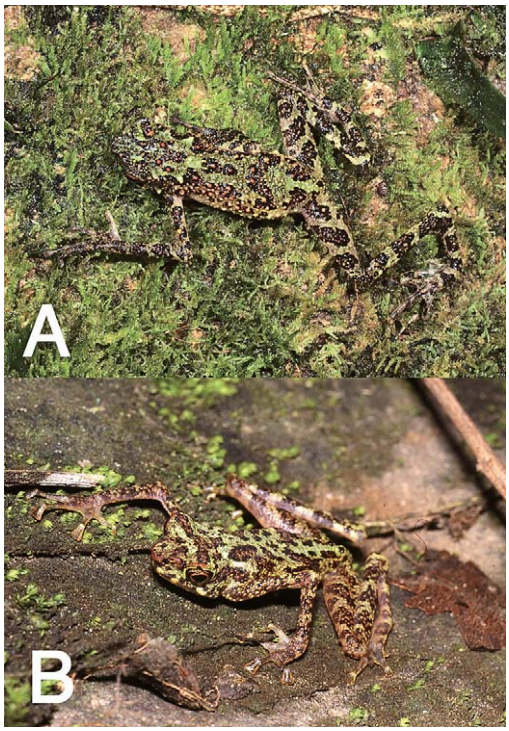

FIG. 7. *Ansonia latidisca* (A) and *Sabahphrynus maculatus* (B), showing their morphological resemblance.

DISCUSSION

Ansonia latidisca is morphologically more similar to *S. maculatus* than to most congeneric species in having a fairly large, slender body, very long, slender forelimbs, large spatulate finger disks, and moss-like body color (Inger, 1966; Inger et al., 2001 [as *A. anotis*]; Matsui et al., 2007: Fig. 7). The two species also resemble one another ecologically. The three specimens of *A. latidisca* were found on a slope far apart from a small stream, on leaves or the trunk of huge trees or on a huge rock, 1.5–2 m above ground. Similarly, some individuals of *S. maculatus* are reported to have been found 10 m apart from the edge of a small stream, 1–2 m above ground on the trunk of a huge tree, mostly in or near a small hole in the trunk, although others were found on a log beside a stream or on a rock at one bank of a stream (Inger et al., 2001; Matsui et al., 2007).

However, the two species decidedly differ by the presence in *A. latidisca* and absence in *S. maculatus* of a distinct tympanum (Fig. 8). Analyses of mitochondrial DNA genes also revealed that the two species are very remote genetically. Since both *A. latidisca* and *Sabahphrynus* are seemingly adapted to arboreal life with similar habitat preferences, their similarity in general morphology is most likely the consequence of convergence.

As reported by Matsui et al. (2010), the genus *Ansonia* is clearly divisible into Clade A from Peninsular Malaysia, Thailand (Subclade A1) and Borneo (Subclade A2), and Clade B from Borneo and the Philippines. Of the two lineages from Borneo, species in Clade A are generally smaller than those in Clade B, and large size of *A. latidisca* suggested its

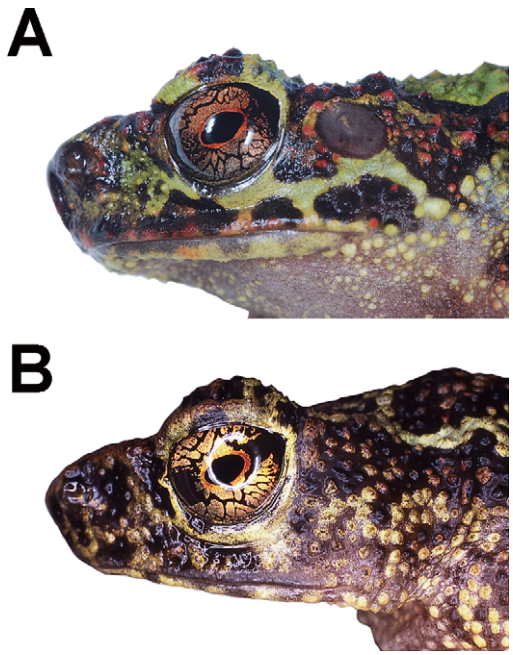

FIG. 8. Lateral views of heads of *Ansonia latidisca* (A) and *Sabahphrynus maculatus* (B), showing the presence in *A. latidisca* and absence in *S. maculatus* of tympanum.

position in Clade B. However, the species was actually basal to the two subclades in Clade A, suggesting its primitive nature within this clade. This finding is significant in suggesting that the genus *Ansonia* originated within Borneo.

Johann Gottfried Hallier, the collector of the holotype of *A. latidisca*, is a German botanist and was an temporary Assistant at the Buitenzorg Herbarium, Java between 1893 and 1896. He traveled to Gunung Damoes, Sambas, Kalimantan, only once between 22 and 24 October 1893 (Van Steenis Kruseman, 2011). Thus the specimen is thought to have been collected during this period. Although detailed data of collection for other specimens are not available, the long absence of collection seems to be partly ascribable to its arboreal habits, probably like *Sabahphrynus maculatus*, which is also arboreal and remained uncollected for nearly 100 years after its initial discovery (Inger, 1966; Inger et al., 2001; Matsui et al., 2007).

Recent intensive surveys revealed that *A. latidisca* is not extinct as once suspected (Stuart et al., 2008), and inhabits at least the primary forest of westernmost Sarawak (Conservation International, 2011). However, the populations are confined to small areas and probably require conservation (Stuart et al., 2008). There still remain many basic issues to be surveyed including breeding habits and larval habitat. From our survey, late February seems to be outside the breeding season, and field surveys in different seasons would be the first step for considering measures of conservation.

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